

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/12, 15/62, C07K 14/705, A61K 38/17</b>		<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 99/58674</b> <b>(43) International Publication Date:</b> 18 November 1999 (18.11.99)
<b>(21) International Application Number:</b> PCT/US99/10588 <b>(22) International Filing Date:</b> 13 May 1999 (13.05.99) <b>(30) Priority Data:</b> 60/085,487 14 May 1998 (14.05.98) US 60/110,836 3 December 1998 (03.12.98) US <b>(71) Applicant (for all designated States except US):</b> IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> ANDERSON, Dirk, M. [US/US]; 3616 N.W. 64th Street, Seattle, WA 98107 (US). GALIBERT, Laurent, J. [US/US]; 617 5th Avenue West, Seattle, WA 98119 (US). <b>(74) Agent:</b> HENRY, Janis, C.; Immunex Corporation, Law Dept., 51 University Street, Seattle, WA 98101 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>	
<b>(54) Title:</b> METHOD OF INHIBITING OSTEOCLAST ACTIVITY			
<b>(57) Abstract</b>  Isolated soluble RANK receptors, DNAs encoding such receptors, and pharmaceutical compositions made therefrom, are disclosed. The isolated receptors can be used to regulate osteoclastogenesis, and hence treat disease in which there is excess bone loss.			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon						

## TITLE

### METHOD OF INHIBITING OSTEOCLAST ACTIVITY

## TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to the field of cytokine receptors, and more specifically to cytokine receptor/ligand pairs having osteoclast regulatory activity.

## BACKGROUND OF THE INVENTION

RANK (Receptor Activator of NF- $\kappa$ B) and its ligand (RANKL) are a recently-described receptor/ligand pair that play an important role in an immune response. The cloning of RANK and RANKL is described in USSN 08/996,139 and USSN 08/995,659, respectively. It has recently been found that RANKL binds to a protein referred to as osteoprotegerin (OPG), a member of the Tumor Necrosis Factor Receptor (TNFR) family. Yasuda et al. (*Proc. Natl. Acad. Sci.* 95:3597; 1998) expression cloned a ligand for OPG, which they referred to as osteoclastogenesis inhibitory factor. Their work was repeated by Lacey et al. (*Cell* 93:165; 1998). In both cases, the ligand they cloned turned out to be identical to RANKL.

In osteoclastogenesis, the interaction of an osteoblast or stromal cell with an osteoclast precursor leads to the differentiation of the precursor into an osteoclast. OPG was known to inhibit this differentiation. A model has been proposed in which RANKL on the osteoblast or stromal cell surface interacts with a specific receptor on an osteoclast progenitor surface, signaling a differentiation event. OPG effectively blocks the interaction of RANKL with a receptor on osteoclast progenitors *in vitro*, and has been shown to ameliorate the effects of ovariectomy on bone-loss in mice. However, OPG is also known to bind other ligands in the TNF family, which may have a deleterious effect on the activities of such ligands *in vivo*. Moreover, the presence of other ligands that bind OPG *in vivo* may require high dosages of OPG to be administered in order to have sufficient soluble OPG available to inhibit osteoclastogenesis.

Accordingly, there is a need in the art to identify soluble factors that specifically bind RANKL and inhibit the ability of RANKL to induce osteoclastogenesis without reacting with other ligands.

30

## SUMMARY OF THE INVENTION

The present invention provides processes associated with the use of a novel receptor, referred to as RANK (for receptor activator of NF- $\kappa$ B), that is a member of the TNF receptor superfamily. RANK is a Type I transmembrane protein having 616 amino acids, consisting of an extracellular domain, transmembrane region and cytoplasmic domain.

triggering of RANK results in the upregulation of the transcription factor NF- $\kappa$ B, a ubiquitous transcription factor that is most extensively utilized in cells of the immune system.

Soluble forms of the receptor can be prepared and used to interfere with signal transduction through membrane-bound RANK. Inhibition of RANKL-mediated signal transduction will be useful in ameliorating the effects of osteoclastogenesis and osteoclast activity in disease conditions in which there is excess bone break down. Examples of such conditions include osteoporosis, Paget's disease, cancers that may metastasize to bone and induce bone breakdown (i.e., multiple myeloma, breast cancer, some melanomas; see also Mundy, C. *Cancer Suppl.* 80:1546; 1997), and cancers that do not necessarily metastasize to bone, but result in hypercalcemia and bone loss (e.g. squamous cell carcinomas).

Soluble forms of RANK comprise the extracellular domain of RANK or a fragment thereof that binds RANKL. Fusion proteins of RANK may be made to allow preparation of soluble RANK. Examples of such fusion proteins include a RANK/Fc fusion protein, a fusion protein of a zipper moiety (i.e., a leucine zipper), and various tags that are known in the art. Other antagonists of the interaction of RANK and RANKL (i.e., antibodies to RANKL, small molecules) will also be useful in the inventive methods. These and other aspects of the present invention will become evident upon reference to the following detailed description of the invention.

### **DETAILED DESCRIPTION OF THE INVENTION**

A novel partial cDNA insert with a predicted open reading frame having some similarity to CD40 was identified and was used to hybridize to colony blots generated from a dendritic cell (DC) cDNA library containing full-length cDNAs. SEQ ID NO:1 shows the nucleotide and amino acid sequence of a predicted full-length protein.

RANK is a member of the TNF receptor superfamily; it most closely resembles CD40 in the extracellular region. RANK is expressed on epithelial cells, some B cell lines, and on activated T cells. However, its expression on activated T cells is late, about four days after activation. This time course of expression coincides with the expression of Fas, a known agent of apoptosis. RANK may act as an anti-apoptotic signal, rescuing cells that express RANK from apoptosis as CD40 is known to do. Alternatively, RANK may confirm an apoptotic signal under the appropriate circumstances, again similar to CD40. RANK and its ligand are likely to play an integral role in regulation of the immune and inflammatory response. The isolation of a DNA encoding RANK is

U.S. Pat. No. 5,881,087, filed December 22, 1997, the disclosure of which is

incorporated by reference herein. USSN 08/996,139 describes several forms of RANK that are useful in the present invention.

Soluble RANK comprises the signal peptide and the extracellular domain (residues 1 to 213 of SEQ ID NO:2) or a fragment thereof. Alternatively, a different  
5 signal peptide can be substituted for the native leader, beginning with residue 1 and continuing through a residue selected from the group consisting of amino acids 24 through 33 (inclusive) of SEQ ID NO:2. Moreover, fragments of the extracellular domain will also provide soluble forms of RANK.

Fragments can be prepared using known techniques to isolate a desired portion of  
10 the extracellular region, and can be prepared, for example, by comparing the extracellular region with those of other members of the TNFR family (of which RANK is a member) and selecting forms similar to those prepared for other family members. Alternatively, unique restriction sites or PCR techniques that are known in the art can be used to prepare numerous truncated forms which can be expressed and analyzed for activity.

Other derivatives of the RANK proteins within the scope of this invention include  
15 covalent or aggregative conjugates of the proteins or their fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-  
20 translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast  $\alpha$ -factor leader).

Protein fusions can comprise peptides added to facilitate purification or  
identification of RANK proteins and homologs (e.g., poly-His). The amino acid sequence of the inventive proteins can also be linked to an identification peptide such as  
25 that described by Hopp et al., *Bio/Technology* 6:1204 (1988; FLAG<sup>TM</sup>). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein.

Fusion proteins further comprise the amino acid sequence of a RANK linked to an  
30 immunoglobulin Fc region. An exemplary Fc region is a human IgG<sub>1</sub> having a nucleotide an amino acid sequence set forth in SEQ ID NO:3. Fragments of an Fc region may also be used, as can Fc muteins. For example, certain residues within the hinge region of an Fc region are critical for high affinity binding to Fc $\gamma$ RI. Canfield and Morrison (*J. Exp. Med.* 173:1483; 1991) reported that Leu(234) and Leu(235) were critical to high affinity  
35 binding of IgG<sub>1</sub> to Fc $\gamma$ RI present on U937 cells. Similar results were obtained by Lund et

for FcR. Depending on the portion of the Fc region used, a fusion protein may be expressed as a dimer, through formation of interchain disulfide bonds. If the fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a protein oligomer with as many as four RANK regions.

5 In another embodiment, RANK proteins further comprise an oligomerizing peptide such as a zipper domain. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988). Zipper domain is a term used to refer to a conserved peptide domain present in these (and other) proteins, which is responsible for multimerization of the proteins. The zipper domain comprises a  
10 repetitive heptad repeat, with four or five leucine, isoleucine or valine residues interspersed with other amino acids. Examples of zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, *fos* and *jun*, also exhibit zipper domains, as does the gene product of the murine proto-  
15 oncogene, *c-myc* (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes *fos* and *jun* comprise zipper domains that preferentially form a heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989). A preferred zipper moiety is that of SEQ ID NO:6 or a fragment thereof. This and other zippers are disclosed in US Patent 5,716,805.

20 Other embodiments of useful proteins include RANK polypeptides encoded by DNAs capable of hybridizing to the DNA of SEQ ID NO:1 under moderately stringent conditions (prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight) to the DNA sequences encoding RANK, or more preferably under stringent conditions (for example, hybridization in 6 X  
25 SSC at 63°C overnight; washing in 3 X SSC at 55°C), and other sequences which are degenerate to those which encode the RANK. In one embodiment, RANK polypeptides are at least about 70% identical in amino acid sequence to the amino acid sequence of native RANK protein as set forth in SEQ ID NO:2 for human RANK and NO:6 for murine RANK. In a preferred embodiment, RANK polypeptides are at least about 80%  
30 identical in amino acid sequence to the native form of RANK; most preferred polypeptides are those that are at least about 90% identical to native RANK.

Percent identity may be determined using a computer program, for example, the GAP computer program described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGC). For  
35 fragments derived from the RANK protein, the identity is calculated based on that portion

of the RANK protein that is present in the fragment

Examples herein. Suitable assays include, for example, an enzyme immunoassay or a dot blot, and assays that employ cells expressing RANKL. Suitable assays also include, for example, inhibition assays, wherein soluble RANK is used to inhibit the interaction of RANKL with membrane-bound or solid-phase associated RANK (i.e., signal transduction assays). Such methods are well known in the art.

RANKL and RANK are important factors in osteoclastogenesis. RANK is expressed on osteoclasts and interacts with RANK ligand (RANKL) to mediate the formation of osteoclast-like (OCL) multinucleated cells. This was shown by treating mouse bone marrow preparations with M-CSF (CSF-1) and soluble RANKL for 7 days in culture. No additional osteoclastogenic hormones or factors were necessary for the generation of the multinucleated cells. Neither M-CSF nor RANKL alone led to the formation of OCL. The multinucleated cells expressed tartrate resistant acid phosphatase and were positive for [<sup>125</sup>I]- calcitonin binding. The tyrosine kinase c-src was highly expressed in multinucleated OCL and a subset of mononuclear cells as demonstrated by immunofluorescence microscopy. (See Example 2).

#### Purification of Recombinant RANK

Purified RANK, and homologs or analogs thereof are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a counter structure protein or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying the inventive proteins.

Affinity chromatography is a particularly preferred method of purifying RANK and homologs thereof. For example, a RANK expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity

zipper domain. Monoclonal antibodies against the RANK protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art. A ligand may also be used to prepare an affinity matrix for affinity purification of RANK.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a RANK composition. Suitable methods include those analogous to the method disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Fermentation of yeast which express the inventive protein as a secreted protein greatly simplifies purification.

Protein synthesized in recombinant culture is characterized by the presence of cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover the inventive protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of the inventive proteins free of other proteins which may be normally associated with the proteins as they are found in nature in the species of origin.

#### Uses and Administration of RANK Compositions

The present invention provides methods of using therapeutic compositions comprising a protein and a suitable diluent and carrier. These methods involve the use of therapeutic compositions of RANK or soluble fragments of RANK for regulating an immune or inflammatory response. Further included within the present invention are methods for regulating osteoclast activity by administering therapeutic compositions of RANK or soluble RANK fragments to an individual in amounts sufficient to decrease excess bone resorption. Typically, the individual is afflicted with excess bone resorption and suffers from the effects of hypercalcemia, has symptoms of hypercalcemia, or is



activity, regulating osteoclast generation and inhibiting osteoclast generation in individuals inflicted with excess bone resorption. In connection with the methods described herein, the present invention contemplates the use of RANK in conjunction with soluble cytokine receptors or cytokines, or other osteoclast/osteoblast regulatory molecules.

In connection with the methods described herein, RANK ligand (RANKL) on osteoblasts or stromal cells is known to interact with RANK on osteoclast progenitor surfaces signaling an event that leads to the differentiation of osteoclast precursors into osteoclasts. (See Example 2 below.) Thus, RANK, and in particular soluble forms of RANK, is useful for the inhibition of the RANKL-mediated signal transduction that leads to the differentiation of osteoclast precursors into osteoclasts. Soluble forms of RANK are also useful for the regulation and inhibition of osteoclast activity, e.g. bone resorption. By interfering with osteoclast differentiation, soluble forms of RANK are useful in the amelioration of the effects of osteoclastogenesis in disease conditions in which there is excess bone break down. Such disease conditions include Paget's disease, osteoporosis, and cancer. Many cancers metastasize to bone and induce bone breakdown by locally disrupting normal bone remodeling. Such cancers can be associated with enhanced numbers of osteoclasts and enhanced amount of osteoclastic bone resorption resulting in hypercalcemia. These cancers include, but are not limited to, breast cancer, multiple myeloma, melanomas, lung cancer, prostate, hematologic, head and neck, and renal. (See Guise et al. *Endocrine Reviews*, 19(1):18-54, 1998.) Soluble forms of RANK can be administered to such cancer patients to disrupt the osteoclast differentiation pathway and result in fewer numbers of osteoclast, less bone resorption, and relief from the negative effects of hypercalcemia.

Other cancers do not metastasize to bone, but are known to act systemically on bone to disrupt bone remodeling and result in hypercalcemia. (See Guise et al. *Endocrine Reviews*, 19(1):18-54, 1998.) In accordance with this invention, RANKL has been found on the surface of certain squamous cells that do not metastasize to bone but are associated with hypercalcemia. (See Example 3 below) Squamous cells that are associated with hypercalcemia also express M-CSF (CSF-1), a cytokine that, together with RANKL, stimulates the proliferation and differentiation of osteoclast precursors to osteoclasts. In accordance with the present invention, it has been discovered that M-CSF directly upregulates RANK on surfaces of osteoclast precursors. When squamous cells release excessive amounts of CSF-1, increased expression of RANK occurs on the surfaces of osteoclast precursors. Thus, there is a higher probability that RANK will interact with RANKL on osteoblasts or stromal cells to produce increased numbers of osteoclasts,

In addition to the ameliorating the effects of cancers that metastasize to bone, the present invention provides methods for ameliorating the systemic effects, e.g. hypercalcemia, of cancers that are associated with excess osteoclast activity (e.g. squamous cell carcinomas). Such methods include administering soluble forms of RANK  
5 in amounts sufficient to interfere with the RANK/RANKL signal transduction that leads to the differentiation of osteoclast precursors into osteoclasts. Fewer osteoclasts lead to reduced bone resorption and relief from the negative effects of hypercalcemia.

For therapeutic use, purified protein is administered to an individual, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example,  
10 RANK protein compositions administered to regulate osteoclast function can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique. Typically, a therapeutic agent will be administered in the form of a composition comprising purified RANK, in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to recipients at the dosages  
15 and concentrations employed.

Ordinarily, the preparation of such protein compositions entails combining the inventive protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other  
20 stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being  
25 treated, the desired response, the condition of the patient, and so forth.

Soluble forms of RANK and other RANK antagonists such as antagonistic monoclonal antibodies can be administered for the purpose of inhibiting RANK-induced osteoclastogenesis. It is desirable to inhibit osteoclastogenesis in various disease states in which excess bone loss occurs. Examples include osteoporosis, Pagett's disease, and  
30 various cancers. Various animal models of these diseases are known in the art; accordingly, it is a matter of routine experimentation to determine optimal dosages and routes of administration of soluble RANK, first in an animal model and then in human clinical trials.

35 The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention

These examples are intended to illustrate the invention and are not to be construed as limiting the scope of the invention. The following examples are intended to illustrate the invention and are not to be construed as limiting the scope of the invention.

### EXAMPLE 1

This example describes a plate binding assay useful in comparing the ability of various ligands to bind receptors. The assay is performed essentially as described in Smith et al., Virology 236:316 (1997). Briefly, 96-well microtiter plates are coated with an antibody to human Fc (i.e., polyclonal goat anti human Fc). Receptor/Fc fusion proteins are then added, and after incubation, the plates are washed. Serial dilutions of the ligands are then added. The ligands may be directly labeled (i.e., with  $^{125}\text{I}$ ), or a detecting reagent that is radioactively labeled may be used. After incubation, the plates are washed, specifically bound ligands are released, and the amount of ligand bound quantified.

Using this method, RANK/Fc and OPG/Fc were bound to 96-well plates. In an indirect method, a RANKL/zipper fusion is detected using a labeled antibody to the zipper moiety. It was found that human OPG/Fc binds mRANKL at 0.05 nM, and human RANK/Fc binds mRANKL at 0.1 nM. These values indicate similar binding affinities of OPG and RANK for RANKL, confirming the utility of RANK as an inhibitor of osteoclast activity in a manner similar to OPG.

### EXAMPLE 2

The following describes the formation of osteoclast like cells from bone marrow cell cultures using a soluble RANKL in the form of soluble RANKL/leucine zipper fusion protein (RANKL LZ).

Using RANKL LZ at  $1\mu\text{g/ml}$ , osteoclasts were generated from murine bone marrow (BM) in the presence of CSF-1. These osteoclasts are formed by the fusion of macrophage-like cells and are characterized by their TRAP (tartrate-resistant acid phosphatase) positivity. No TRAP<sup>+</sup> cells were seen in cultures containing CSF-1 alone or in cultures containing CSF-1 and TRAIL LZ (a control for the soluble RANKL LZ). Even though human and monkey bone marrow contains more contaminating fibroblasts than murine bone marrow, osteoclasts were generated from murine and monkey bone marrow with the combination of CSF-1 and soluble RANKL LZ. In a dose-response study using murine bone marrow and suboptimal amounts of CSF-1 (40ng/ml), the effects of soluble RANKL LZ plateaued at about 100ng/ml.

The effect of soluble RANKL LZ on proliferation of cells was studied in the same cultures using Alamar Blue. After 5 days, the proliferative response was lower in cultures containing CSF-1 and RANKL LZ than in those containing CSF-1 alone. The supports these observations that soluble RANKL LZ is inducing osteoclast differentiation. When

survive if recultured in CSF-1. When RANKL LZ was added to these cultures there was no added benefit. Thus, the combination of CSF-1 and RANKL are required for the generation of osteoclast. Additionally, once formed, CSF-1 is sufficient to maintain their survival in culture.

5           Finally, using human bone marrow, soluble anti-human RANK mAb and immobilized anti-human RANK mAb were compared to RANKL LZ for the generation of osteoclasts in the presence of CSF-1. Immobilized M331 and RANKL LZ were found to be equally effective for osteoclast generation while soluble M331 was superior to both immobilized antibody and RANKL LZ. This confirms that the osteoclast differentiating  
10 activity of RANKL is mediated through RANK rather than via an alternative receptor.

          Since osteoclasts cannot readily be harvested and analyzed by flow cytometry, <sup>125</sup>I-labeled calcitonin binding assays were used to identify osteoclasts (the calcitonin receptor is considered to be an osteoclast-specific marker). Osteoclasts generated from murine BM cultured with CSF-1 and RANKL LZ for 9 days showed binding of  
15 radiolabeled calcitonin confirming their osteoclast identity.

### **EXAMPLE 3**

          In order to determine RANKL expression by either of two different squamous cell carcinomas, standard Western blot and RT-PCR studies were performed on MH-85 and  
20 OKK cells. One of these carcinoma cells, the MH-85 cells, is associated with hypercalcemia.

          The results confirmed that MH-85 and OKK squamous cells express RANKL. MH-85 cells, in addition to being linked with hypercalcemia in patients inflicted with this carcinoma, also express M-CSF (CSF-1). It was also determined that CSF-1 upregulates  
25 RANK expression on osteoclast precursors. The enhanced amount of CSF-1 in MH-85 type squamous cell cancer patients can lead to an upregulation of RANK and increased RANK interaction with RANKL. Signals transduced by RANK and RANKL interaction result in increased numbers of mature osteoclasts and bone breakdown. Since soluble forms of RANK can inhibit the RANK/RANKL interaction, administering a soluble form  
30 of RANK (e.g. the extracellular region of RANK fused to an Fc) to a squamous cell cancer patient provides relief from adverse effects of this cancer, including hypercalcemia.

35

## CLAIMS

We claim:

1. A method of regulating osteoclast activity, the method comprising causing a soluble RANK to bind RANKL.
2. The method of claim 1, wherein the soluble RANK is encoded by a DNA selected from the group consisting of:
  - (a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:62, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;
  - (b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;
  - (c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode RANK polypeptides that bind RANKL; and
  - (d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c), wherein the fragments of RANK polypeptides bind RANKL.
3. The method of claim 2, wherein the RANK is at least about 80% identical in amino acid sequence to native RANK
4. The method of claim 3, wherein the RANK further comprises a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG™ tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.
5. A method of ameliorating effects of excess bone loss, comprising administering a soluble RANK polypeptide composition to an individual at risk for excess bone loss, and allowing the soluble RANK to bind RANKL and inhibit binding thereof to cells expressing RANK.

6. The method of claim 5, wherein the individual is at risk from or suffers from a condition selected from the group consisting of osteoporosis, Pagett's disease, and bone cancer, and cancers associated with hypercalcemia.

7. The method of claim 5, wherein the soluble RANK is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:62, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode RANK polypeptides that bind RANKL; and

(d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c), wherein the fragments of RANK polypeptides bind RANKL.

8. The method of claim 7, wherein the RANK is at least about 80% identical in amino acid sequence to native RANK

9. The method of claim 8, wherein the RANK further comprises a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG<sup>TM</sup> tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

10. The method of claim 6, wherein the soluble RANK is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:62, and a carboxy terminus selected from the group consisting an amino acid between amino acid

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode RANK polypeptides that bind RANKL; and

(d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c), wherein the fragments of RANK polypeptides bind RANKL.

11. The method of claim 10, wherein the RANK is at least about 80% identical in amino acid sequence to native RANK

12. The method of claim 11, wherein the RANK further comprises a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG<sup>TM</sup> tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Immunex Corporation  
Anderson, Dirk M.  
Galibert, Laurent
- (ii) TITLE OF INVENTION: METHOD OF INHIBITING OSTEOCLAST ACTIVITY
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Immunex Corporation, Law Department
  - (B) STREET: 51 University Street
  - (C) CITY: Seattle
  - (D) STATE: WA
  - (E) COUNTRY: USA
  - (F) ZIP: 98101
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM Compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #2.0
- (vi) CURRENT APPLICATION DATA:
  - (A) INT'L APPLICATION NUMBER: --to be assigned--
  - (B) FILING DATE: 13 May 1999
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Henry, Janis C.
  - (B) REGISTRATION NUMBER: 34,347
  - (C) REFERENCE/DOCKET NUMBER: 2874-WO
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: (206)587-0430
  - (B) TELEFAX: (206)233-0644

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 3136 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:



(B) CLONE: FULL LENGTH RANK

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 39..1886

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGCTGAGGC CGCGGCGCCC GCCAGCCTGT CCCGCGCC	ATG GCC CCG CGC GCC	53
	Met Ala Pro Arg Ala	
	1 5	
CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTC TGC GCG CTG CTC		101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Cys Ala Leu Leu		
	10 15 20	
GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG		149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu		
	25 30 35	
AAG CAT TAT GAG CAT CTG GCA CGG TGC TGT AAC AAA TGT GAA CCA GGA		197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly		
	40 45 50	
AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG		245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu		
	55 60 65	
CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA		293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys		
	70 75 80 85	
TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC CTG		341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val		
	90 95 100	
GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG		389
Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly		
	105 110 115	
TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC		437
Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys		
	120 125 130	
GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA		485
Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr		
	135 140 145	
CTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC		533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser		
	150 155 160 165	
ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA		581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg		
	170 175 180	
GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT		629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser		
	185 190 195	

TTA ATA ATT CTG CTT CTC TTC GCG TCT GTG GCC CTG GTG GCT GCC ATC Leu Ile Ile Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala Ile 215 220 225	725
ATC TTT GGC GTT TGC TAT AGG AAA AAA GGG AAA GCA CTC ACA GCT AAT Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn 230 235 240 245	773
TTG TGG CAC TGG ATC AAT GAG GCT TGT GGC CGC CTA AGT GGA GAT AAG Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys 250 255 260	821
GAG TCC TCA GGT GAC AGT TGT GTC AGT ACA CAC ACC GCA AAC TTT GGT Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly 265 270 275	869
CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG ACT CTG GAG GAG AAG Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys 280 285 290	917
ACA TTT CCA GAA GAT ATG TCC TAC CCA GAT CAA GGT GGT GTC TGT CAG Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln Gly Gly Val Cys Gln 295 300 305	965
GGC ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA GGC GAA GAT GCC AGG Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg 310 315 320 325	1013
ATG CTC TCA TTG GTC AGC AAG ACC GAG ATA GAG GAA GAC AGC TTC AGA Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg 330 335 340	1061
CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG CCC TCC CAG CCC ACA Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr 345 350 355	1109
GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC AAA TCC ACA CCT CCT Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro 360 365 370	1157
TTC TCT GAA CCC CTG GAG GTG GGG GAG AAT GAC AGT TTA AGC CAG TGC Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln Cys 375 380 385	1205
TTC ACG GGG ACA CAG AGC ACA GTG GGT TCA GAA AGC TGC AAC TGC ACT Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr 390 395 400 405	1253
GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG TCC TCT GAA AAC TAC Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn Tyr 410 415 420	1301
TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG CAC TGG GCA GCC AGC Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro His Trp Ala Ala Ser 425 430 435	1349
CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC CCT CCT Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro Pro 440 445 450	1397

CCC CAG TGC GCC TAT GGC ATG GGC CTT CCC CCT GAA GAA GAA GCC AGC Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro Glu Glu Glu Ala Ser 470 475 480 485	1493
AGG ACG GAG GCC AGA GAC CAG CCC GAG GAT GGG GCT GAT GGG AGG CTC Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly Ala Asp Gly Arg Leu 490 495 500	1541
CCA AGC TCA GCG AGG GCA GGT GCC GGG TCT GGA AGC TCC CCT GGT GGC Pro Ser Ser Ala Arg Ala Gly Ala Ser Gly Ser Ser Pro Gly Gly 505 510 515	1589
CAG TCC CCT GCA TCT GGA AAT GTG ACT GGA AAC AGT AAC TCC ACG TTC Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr Phe 520 525 530	1637
ATC TCC AGC GGG CAG GTG ATG AAC TTC AAG GGC GAC ATC ATC GTG GTC Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val Val 535 540 545	1685
TAC GTC AGC CAG ACC TCG CAG GAG GGC GCC CCG GCG GCT GCG GAG CCC Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala Ala Ala Glu Pro 550 555 560 565	1733
ATG GGC CGC CCG GTG CAG GAG GAG ACC CTG GCG CGC CGA GAC TCC TTC Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala Arg Arg Asp Ser Phe 570 575 580	1781
GCG GGG AAC GGC CCG CGC TTC CCG GAC CCG TGC GGC GGC CCC GAG GGG Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu Gly 585 590 595	1829
CTG CGG GAG CCG GAG AAG GCC TCG AGG CCG GTG CAG GAG CAA GGC GGG Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly Gly 600 605 610	1877
GCC AAG GCT TGAGCGCCCC CCATGGCTGG GAGCCCCAAG CTCGGAGCCA Ala Lys Ala 615	1926
GGGCTCGCGA GGGCAGCACC GCAGCCTCTG CCCAGCCCC GGCCACCCAG GGATCGATCG	1986
GTACAGTCCA GGAAGACCAC CCGGCATTCT CTGCCCCATT TGCCTTCCAG GAAATGGGCT	2046
TTTCAGGAAG TGAATTGATG AGGACTGTCC CCATGCCAC GGATGCTCAG CAGCCCCGCC	2106
CACTGGGGCA GATGTCTCCC CTGCCACTCC TCAAACCTCG AGCAGTAATT TGTGGCACTA	2166
TGACAGCTAT TTTTATGACT ATCCTGTTCT GTGGGGGGGG GGTCTATGTT TTCCCCCAT	2226
ATTTGTATTC CTTTTCATAA CTTTCTTGA TATCTTTCCT CCCTCTTTT TAATGTAAAG	2286
GTTTTCTCAA AAATCTCCT AAAGGTGAGG GTCTCTTCT TTTCTTTTT CTTTTTTTT	2346
TTCTTTTTTT GGCAACCTGG CTCTGGCCCC GGCTAGAGTG CAGTGGTGCG ATTATAGCCC	2406
GGTGCAGCCT CTAACCTCTG GCCTCAAGCA ATCCAAGTGA TCCTCCACCC TCAACCTTCG	2466
GACTAGCTGG GATCAGAGCT GCAGGCCACG CCCAGCTTCC TCCCCCGAC TCCCCCCCC	2526

```

TTTACGTATT TTCTTTTGTG CCCCTGCTCA CAGTGTMTTA GAGATGGCTT TCCCAGTGTG      2706
TGTTCAATTGT AAACACTTTT GGGAAAGGGC TAAACATGTG AGGCCTGGAG ATAGTTGCTA      2766
AGTTGCTAGG AACATGTGGT GGGACTTTCA TATTCTGAAA AATGTTCTAT ATTCTCATTT      2826
TTCTAAAAGA AAGAAAAAAG GAAACCCGAT TTATTTCTCC TGAATCTTTT TAAGTTTGTG      2886
TCGTTCCCTTA AGCAGAACTA AGCTCAGTAT GTGACCTTAC CCGCTAGGTG GTTAATTTAT      2946
CCATGCTGGC AGAGGCACTC AGGTACTTGG TAAGCAAATT TCTAAAACCTC CAAGTTGCTG      3006
CAGCTTGGCA TTCTTCTTAT TCTAGAGGTC TCTCTGAAA AGATGGAGAA AATGAACAGG      3066
ACATGGGGCT CCTGGAAAGA AAGGGCCCGG GAAGTTCAAG GAAGAATAAA GTTGAAATTT      3126
TAAAAA      3136

```

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 616 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ala Pro Arg Ala Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu
 1             5             10             15
Leu Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro
 20             25             30
Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
 35             40             45
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
 50             55             60
Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
 65             70             75             80
Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
 85             90             95
Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
100             105             110
Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
115             120             125
Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
130             135             140
Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
145             150             155             160

```

Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala  
 180 185 190  
 Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His  
 195 200 205  
 Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala  
 210 215 220  
 Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys  
 225 230 235 240  
 Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg  
 245 250 255  
 Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His  
 260 265 270  
 Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu  
 275 280 285  
 Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln  
 290 295 300  
 Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln  
 305 310 315 320  
 Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu  
 325 330 335  
 Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg  
 340 345 350  
 Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser  
 355 360 365  
 Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp  
 370 375 380  
 Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu  
 385 390 395 400  
 Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met  
 405 410 415  
 Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro  
 420 425 430  
 His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly  
 435 440 445  
 Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro  
 450 455 460  
 Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro  
 465 470 475 480  
 Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly  
 485 490 495  
 Ser Gly

Ser Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn  
 515 520 525

Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly  
 530 535 540

Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala  
 545 550 555 560

Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala  
 565 570 575

Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys  
 580 585 590

Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val  
 595 600 605

Gln Glu Gln Gly Gly Ala Lys Ala  
 610 615

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 232 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: not relevant
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Human
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: IgG1 Fc mutein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala  
 1 5 10 15

Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
 20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
 35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val  
 50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
 65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
 85 90 95

Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala  
 100 110

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr  
 130 135 140  
 Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg  
 145 150 155 160  
 His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
 165 170 175  
 Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
 180 185 190  
 Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe  
 195 200 205  
 Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
 210 215 220  
 Ser Leu Ser Leu Ser Pro Gly Lys  
 225 230

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1878 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Murine
- (vii) IMMEDIATE SOURCE:
  - (A) LIBRARY: Murine Fetal Liver Epithelium
  - (B) CLONE: muRANK
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 1..1875

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATG	GCC	CCG	CGC	GCC	CGG	CGG	CGC	CGC	CAG	CTG	CCC	GCG	CCG	CTG	CTG	48
Met	Ala	Pro	Arg	Ala	Arg	Arg	Arg	Arg	Gln	Leu	Pro	Ala	Pro	Leu	Leu	
1				5				10						15		
CGG	CTC	TGC	GTG	CTG	CTC	GTT	CCA	CTG	CAG	GTG	ACT	CTC	CAG	GTC	ACT	96
Ala	Leu	Cys	Val	Leu	Leu	Val	Pro	Leu	Gln	Val	Thr	Leu	Gln	Val	Thr	
			20					25						30		

AGC AGA TGC GAA CCA GGA AAG TAC CTG TCC TCT AAG TGC ACT CCT ACC 192  
 Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr  
 50 55 60

TCC GAC AGT GTG TGT CTG CCC TGT GGC CCC GAT GAG TAC TTG GAC ACC 240  
 Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr  
 65 70 75 80

TGG AAT GAA GAA GAT AAA TGC TTG CTG CAT AAA GTC TGT GAT GCA GGC 288  
 Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly  
 85 90 95

AAG GCC CTG GTG GCG GTG GAT CCT GGC AAC CAC ACG GCC CCG CGT CGC 336  
 Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg  
 100 105 110

TGT GCT TGC ACG GCT GGC TAC CAC TGG AAC TCA GAC TGC GAG TGC TGC 384  
 Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys  
 115 120 125

CGC AGG AAC ACG GAG TGT GCA CCT GGC TTC GGA GCT CAG CAT CCC TTG 432  
 Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu  
 130 135 140

CAG CTC AAC AAG GAT ACG GTG TGC ACA CCC TGC CTC CTG GGC TTC TTC 480  
 Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe  
 145 150 155 160

TCA GAT GTC TTT TCG TCC ACA GAC AAA TGC AAA CCT TGG ACC AAC TGC 528  
 Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys  
 165 170 175

ACC CTC CTT GGA AAG CTA GAA GCA CAC CAG GGG ACA ACG GAA TCA GAT 576  
 Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp  
 180 185 190

GTG GTC TGC AGC TCT TCC ATG ACA CTG AGG AGA CCA CCC AAG GAG GCC 624  
 Val Val Cys Ser Ser Ser Met Thr Leu Arg Arg Pro Pro Lys Glu Ala  
 195 200 205

CAG GCT TAC CTG CCC AGT CTC ATC GTT CTG CTC CTC TTC ATC TCT GTG 672  
 Gln Ala Tyr Leu Pro Ser Leu Ile Val Leu Leu Phe Ile Ser Val  
 210 215 220

GTA GTA GTG GCT GCC ATC ATC TTC GGC GTT TAC TAC AGG AAG GGA GGG 720  
 Val Val Val Ala Ala Ile Ile Phe Gly Val Tyr Tyr Arg Lys Gly Gly  
 225 230 235 240

AAA GCG CTG ACA GCT AAT TTG TGG AAT TGG GTC AAT GAT GCT TGC AGT 768  
 Lys Ala Leu Thr Ala Asn Leu Trp Asn Trp Val Asn Asp Ala Cys Ser  
 245 250 255

AGT CTA AGT GGA AAT AAG GAG TCC TCA GGG GAC CGT TGT GCT GGT TCC 816  
 Ser Leu Ser Gly Asn Lys Glu Ser Ser Gly Asp Arg Cys Ala Gly Ser  
 260 265 270

CAC TCG GCA ACC TCC AGT CAG CAA GAA GTG TGT GAA GGT ATC TTA CTA 864  
 His Ser Ala Thr Ser Ser Gln Gln Glu Val Cys Glu Gly Ile Leu Leu  
 275 280 285



GGG CCT GTG TGT GCG GCA GGT GGG CCC TGG GCA GAA GTC AGA GAT TCT	960
Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser	
305 310 315 320	
AGG ACG TTC ACA CTG GTC AGC GAG GTT GAG ACG CAA GGA GAC CTC TCG	1008
Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser	
325 330 335	
AGG AAG ATT CCC ACA GAG GAT GAG TAC ACG GAC CGG CCC TCG CAG CCT	1056
Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro	
340 345 350	
TCG ACT GGT TCA CTG CTC CTA ATC CAG CAG GGA AGC AAA TCT ATA CCC	1104
Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro	
355 360 365	
CCA TTC CAG GAG CCC CTG GAA GTG GGG GAG AAC GAC AGT TTA AGC CAG	1152
Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln	
370 375 380	
TGT TTC ACC GGG ACT GAA AGC ACG GTG GAT TCT GAG GGC TGT GAC TTC	1200
Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe	
385 390 395 400	
ACT GAG CCT CCG AGC AGA ACT GAC TCT ATG CCC GTG TCC CCT GAA AAG	1248
Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys	
405 410 415	
CAC CTG ACA AAA GAA ATA GAA GGT GAC AGT TGC CTC CCC TGG GTG GTC	1296
His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val	
420 425 430	
AGC TCC AAC TCA ACA GAT GGC TAC ACA GGC AGT GGG AAC ACT CCT GGG	1344
Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly	
435 440 445	
GAG GAC CAT GAA CCC TTT CCA GGG TCC CTG AAA TGT GGA CCA TTG CCC	1392
Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro	
450 455 460	
CAG TGT GCC TAC AGC ATG GGC TTT CCC AGT GAA GCA GCA GCC AGC ATG	1440
Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met	
465 470 475 480	
GCA GAG GCG GGA GTA CGG CCC CAG GAC AGG GCT GAT GAG AGG GGA GCC	1488
Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala	
485 490 495	
TCA GGG TCC GGG AGC TCC CCC AGT GAC CAG CCA CCT GCC TCT GGG AAC	1536
Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn	
500 505 510	
GTG ACT GGA AAC AGT AAC TCC ACG TTC ATC TCT AGC GGG CAG GTG ATG	1584
Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met	
515 520 525	
AAC TTC AAG GGT GAC ATC ATC GTG GTG TAT GTC AGC CAG ACC TCG CAG	1632
Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln	
530 535 540	
GGG GGT GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG	1680

GTG CAG GAG GAG ACG CTG GCA CAC AGA GAC TCC TTT GCG GGC ACC GCG 1728  
 Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala  
 565 570 575  
 CCG CGC TTC CCC GAC GTC TGT GCC ACC GGG GCT GGG CTG CAG GAG CAG 1776  
 Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln  
 580 585 590  
 GGG GCA CCC CGG CAG AAG GAC GGG ACA TCG CGG CCG GTG CAG GAG CAG 1824  
 Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln  
 595 600 605  
 GGT GGG GCG CAG ACT TCA CTC CAT ACC CAG GGG TCC GGA CAA TGT GCA 1872  
 Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala  
 610 615 620  
 GAA TGA 1878  
 Glu  
 625

## (2) INFORMATION FOR SEQ ID NO.5.

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 625 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ala Pro Arg Ala Arg Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu  
 1 5 10 15  
 Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr  
 20 25 30  
 Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys  
 35 40 45  
 Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr  
 50 55 60  
 Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr  
 65 70 75 80  
 Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly  
 85 90 95  
 Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg  
 100 105 110  
 Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys  
 115 120 125  
 Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu  
 130 135 140  
 Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe  
 145 150 155 160

Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp  
 180 185 190  
 Val Val Cys Ser Ser Ser Met Thr Leu Arg Arg Pro Pro Lys Glu Ala  
 195 200 205  
 Gln Ala Tyr Leu Pro Ser Leu Ile Val Leu Leu Leu Phe Ile Ser Val  
 210 215 220  
 Val Val Val Ala Ala Ile Ile Phe Gly Val Tyr Tyr Arg Lys Gly Gly  
 225 230 235 240  
 Lys Ala Leu Thr Ala Asn Leu Trp Asn Trp Val Asn Asp Ala Cys Ser  
 245 250 255  
 Ser Leu Ser Gly Asn Lys Glu Ser Ser Gly Asp Arg Cys Ala Gly Ser  
 260 265 270  
 His Ser Ala Thr Ser Ser Gln Gln Glu Val Cys Glu Gly Ile Leu Leu  
 275 280 285  
 Met Thr Arg Glu Glu Lys Met Val Pro Glu Asp Gly Ala Gly Val Cys  
 290 295 300  
 Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser  
 305 310 315 320  
 Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser  
 325 330 335  
 Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro  
 340 345 350  
 Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro  
 355 360 365  
 Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln  
 370 375 380  
 Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe  
 385 390 395 400  
 Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys  
 405 410 415  
 His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val  
 420 425 430  
 Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly  
 435 440 445  
 Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro  
 450 455 460  
 Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met  
 465 470 475 480  
 Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala  
 485 490 495

Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met  
 515 520 525

Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln  
 530 535 540

Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro  
 545 550 555 560

Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala  
 565 570 575

Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln  
 580 585 590

Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln  
 595 600 605

Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala  
 610 615 620

Glu  
 625

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 33 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile  
 1 5 10 15

Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu  
 20 25 30

Arg